Fabrication and surface modification of macroporous poly(L-lactic acid) and poly(L-lactic-*co*-glycolic acid) (70/30) cell scaffolds for human skin fibroblast cell culture

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Abstract: The fabrication and surface modification of a porous cell scaffold are very important in tissue engineering. Of most concern are high-density cell seeding, nutrient and oxygen supply, and cell affinity. In the present study, poly(L-lactic acid) and poly(L-lactic-*co*-glycolic acid) (70/30) cell scaffolds with different pore structures were fabricated. An improved method based on Archimedes' Principle for measuring the porosity of scaffolds, using a density bottle, was developed. Anhydrous ammonia plasma treatment was used to modify surface properties to improve the cell affinity of the scaffolds. The results show that hydrophilicity and surface energy were improved. The polar N-containing groups and positive charged groups also were incorporated into the sample surface. A low-temperature treatment was used to maintain the plasma-modified surface properties effectively. It would do help to the further application of plasma treatment technique. Cell culture results showed that pores smaller than 160 μm are suitable for human skin fibroblast cell growth. Cell seeding efficiency was maintained at above 99%, which is better than the efficiency achieved with the common method of prewetting by ethanol. The plasma-treatment method also helped to resolve the problem of cell loss during cell seeding, and the negative effects of the ethanol trace on cell culture were avoided. The results suggest that anhydrous ammonia plasma treatment enhances the cell affinity of porous scaffolds. Mass transport issues also have been considered. © 2002 Wiley Periodicals, Inc. J Biomed Mater Res 62: 438–446, 2002

Key words: polylactone; surface modification; ammonia plasma treatment; human skin fibroblast cells; tissue engineering

INTRODUCTION

Tissue engineering using cell transplantation appears to be the most promising alternative to existing therapies for restoring tissue and organ function.[1–8] Significant challenges to this technique include the design and fabrication of a suitable biodegradable cell scaffold that can promote cell adhesion, support cell growth, proliferation, and differentiation, and guide the process of tissue development. Transport issues are very important for a tissue engineering scaffold and include nutrient delivery, waste removal, exclusion of materials or cells, protein transport, and cell migration, which, in turn, are governed by the scaffold's porous structure.[9] Pore size, porosity, and the overall continuity of the pores generally are used to characterize pore structure. Scaffolds with pore sizes over 50 nm are called macroporous scaffolds and often are used when an integrated tissue structure is desirable.

There are several methods used to fabricate highly porous biodegradable polymer cell scaffolds, including particulate-leaching,[10–12] phase separation,[13–15] gas foaming,[16,17] emulsion freeze drying,[18] and 3-D printing techniques.[19] These methods can be used to fabricate a sponge-like scaffold, which then can be laminated into three-dimensional foams[10] or formed into more complex architectures known as superstructures.[20]

The particulate-leaching technique is a convenient method for forming sponge-like scaffolds, and pore size and porosity easily can be controlled with this

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technique. The diameter of cells in suspension dictates the minimum pore size, which varies from one cell type to another. The size of stretched cells also varies from one cell type to another. For different cell types, there are suitable pore sizes to accommodate the different cells to stretch and to growth. It has been reported that pore size should be close to 20 μm for the ingrowth of hepatocytes, from 20–150 μm for skin regeneration, and in the range of 100–150 μm for bone regeneration.[21]

Biodegradable polymers, such as poly(L-lactic acid) (PLLA) and poly(lactic-*co*-glycolic acid) (PLGA), often are utilized to construct cell scaffolds for tissue engineering use. However, the hydrophobicity of these scaffolds inhibits the cells from penetrating into the pores. In addition, there are no cell recognition sites on the surface of this kind of polylactone scaffold, which affects its cell affinity.

Many efforts have been directed towards modifying surface properties such as hydrophilicity/hydrophobicity,[22] surface energy,[23] surface charge,[24,25] surface roughness,[26] and towards improving the cell affinity of the polymer. The plasma technique is a convenient method for modifying surface properties of a material. There is an advantage to using the plasma technique when treating surfaces of complex shape because the plasma treatment is conducted in vacuum and it tends to be pervasive.[27] The plasma technique also easily can be used to introduce the desired groups or chains onto the surface of a material,[28–33] and therefore it is a valuable method for improving the cell affinity of a cell scaffold.

The purpose of the present study was to fabricate sponge-like cell scaffolds of different pore sizes by a particulate-leaching technique and to investigate which kinds of pore structure are suitable for culturing human skin fibroblast cells. An improved method is proposed for measuring the porosity of the scaffold. Plasma techniques improve cell affinity and resolve the problem of the loss of cells when cell seeding. Issues of mass transport also are considered.

MATERIALS AND METHODS

Polymer synthesis[34]

L-lactide (LA) and glycolide (GA) were synthesized from L-lactic acid (Yierbao Lactic Acid Factory, Shanghai) and glycolic acid (Xizhong Chemical Factory, Beijing). PLLA (M_w = 130,000, M_w/M_n = 1.57, measured by gel permeation chromatography; Waters 510 with Shodex KF-800 Columns) and PLGA70/30 (M_w = 94,000, M_w/M_n = 1.55) was synthesized under high vacuum in the presence of stannous octoate as the catalyst (0.05 wt %) at 140° for 12 h and 180° for 20 h, respectively. The PLLA and PLGA were purified by dissolution of the polymer in chloroform and precipitation in ethanol, followed by drying in vacuum at room temperature for 48 h.

Scaffold fabrication[12]

PLLA and PLGA (70/30) were dissolved into dichloromethane, and then the sieved porogen salt (<125, 125–200, 200–280, 280–450, and 450–900 μm) was added into the polylactone solution (porogen weight fraction, 91.7%). The produced mixture was cast into a foursquare poly(tetrafluoroethylene) mold (5 × 5 cm). After solvent evaporation for 48 h, the porogen in the foursquare composite was washed out by changing the water every 6 h for a total of 72 h. It then was air dried for 24 h and vacuum dried at 133 Pa for 48 h to obtain the sponge-like scaffold. The produced scaffolds were stored in a desiccator under vacuum before use. The thickness of the scaffold was 1.48 mm.

Scaffold characterization

The cross sections of scaffolds of different pore size were observed by scanning electron microscopy (SEM, Hitachi S-530), and the images of SEM were analyzed by image analysis software (Yalien company, Beijing) to obtain the pore size data. Over thirty results were averaged to get the mean value and standard deviation of the pore size.

The porosity of the scaffolds was measured using the Archimedes' Principle. In this improved method, a density bottle instead of graduated cylinder[35] was used to measure the density and porosity of a scaffold. Ethanol (density ρ_e) was used as the displacement liquid and operated at 30°C. A density bottle filled with ethanol was weighed (W_1). A scaffold sample of weight W_S was immersed into the density bottle, and the air bubbles in the scaffold pores were evacuated under vacuum. Then the density bottle was supplemented with ethanol to full and weighed (W_2). The scaffold saturated with ethanol was taken out of the density bottle and then the density bottle was weighed (W_3).

The followed parameters of a scaffold were calculated: the volume of the scaffold pore (V_p), the volume of the scaffold skeleton (V_s), the density (ρ_s), and the porosity (ε). A new formula for the volume–mass index (V_p/W_s) was proposed for evaluating the scaffolds:

$$V_p = (W_2 - W_3 - W_S)/\rho_e$$
$$V_S = (W_1 - W_2 + W_S)/\rho_e$$
$$\rho_S = W_S/V_S = W_S\rho_e/(W_1 - W_2 + W_S)$$
$$\varepsilon = V_p/(V_p + V_S) = (W_2 - W_3 - W_S)/(W_1 - W_3).$$

Surface modification

According to a previous study,[36] ammonia plasma treatment can more easily improve the surface hydrophilicity of a material than can other gas plasma treatments. Also the amine group can be introduced onto the sample surface, which can be positively charged at physiologic pH because of the protonation in culture medium. Therefore, the porous scaffolds were modified by anhydrous ammonia plasma treatment, which was carried on Samco Plasma Deposition (Model PD-2, 13.56 MH_z).

The scaffolds were located on the lower electrode. The

chamber was evacuated to less than 10 Pa before it was filled with anhydrous ammonia. After the pressure of the chamber had stabilized to a proper value by venting anhydrous ammonia, glow-discharged plasma was created by controlling the electrical power for 120 s. Then the plasma-treated films were further exposed to ammonia gas for another 10 min, with the treating pressure being maintained after the power was turned off. The modified scaffolds were sealed and stored at 0–4°C before use.

Hydrophilicity and surface energy determination

The transparent PLLA and PLGA (70/30) films (0.10 mm thick) were obtained by a solution-casting method. The contact angles of PLLA and PLGA (70/30) were measured on the air surface of the samples before and after modification using a FACE CA-D type contact angle meter (Kyowa Kaimenkagaku Co., Ltd.). Ten independent determinations at different sites of three samples treated under the same conditions were averaged. Deionized water and diiodomethane were used for the measurement. The surface energy was calculated according to the Harmonic mean equations using Matlab software and expressed as follows:

$$(1 + \cos\theta_1)\,\gamma_1 = 4((\gamma_1{}^d\gamma_s{}^d/(\gamma_1{}^d + \gamma_s{}^d) + \gamma_1{}^p\gamma_s{}^p/(\gamma_1{}^p + \gamma_s{}^p))$$

$$(1 + \cos\theta_2)\,\gamma_2 = 4((\gamma_2{}^d\gamma_s{}^d/(\gamma_2{}^d + \gamma_s{}^d) + \gamma_2{}^p\gamma_s{}^p/(\gamma_2{}^p + \gamma_s{}^p))$$

where γ^d is the dispersive component; γ^p is the polar component; θ_1 is the contact angle to water; and θ_2 is the contact angle to diiodomethane. For water, $\gamma_1 = 72.8$ mJ/m^2; $\gamma_1{}^d =$ 22.1 mJ/m^2; and $\gamma_1{}^p = 50.7$ mJ/m^2. For diiodomethane, $\gamma_2 =$ 50.8 mJ/m^2; $\gamma_2{}^d = 44.1$ mJ/m^2; and $\gamma_2{}^p = 6.7$mJ/m^2.

X-ray photoelectron spectroscopy (XPS) surface analysis

XPS spectra of the samples were acquired on a VG Escalab 220i-xl spectrometer using AlK_α radiation at a power of 300 w. A take-off angle of 90° with respect to the sample's surface was used. The high-resolution spectra C_{1s} and N_{1s} were deconvoluted and curve-fit to analyze the chemical bonding state. All measurements were taken under vacuum (2×10^{-7} Pa).

Cell seeding and MTT assay

Human skin fibroblast cells were separated from skin tissue of human abortion neofetal, age 5 months, by selective attachment culture. The separated skin fibroblast cells were grown in 50-mL cell-culture flasks with Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco), 0.05 mg/mL of ascorbic acid, 0.3 mg/mL of L-glutamine (Gibco), 3.7 mg/mL of $NaHCO_3$ (AR grade, Beijing Chemical Factory), and 100 Units/mL each of penicillin and streptomycin. Cell culture was maintained in a gas-jacket incubator equilibrated with 5% CO_2 at 37°C.

Porous scaffolds were cut into small disks (15 mm in diameter) with the aid of a cork borer in order to locate the disks into the 24-well tissue culture plate. One group of disks was prewetted in 75% ethanol for 2 h, and then the ethanol was exchanged with an excess amount of phosphate-buffered saline (PBS).[37] The PBS was removed from the disks with a pipette. Then the disks were sterilized by Ultraviolet light for 2 h. Another group of disks was pretreated by NH_3 plasma treatment, as described above, and then transferred to an ultracleaning workstation for ultraviolet lighting for 2 h. A cell suspension (100 μL) with a cell density of 3–5 × 10^5 cells/disk was seeded evenly into the scaffolds with a micro-pipette.

The cell-seeded scaffolds were maintained at 37°C under 5% CO_2 for 3 h, and then 2 mL of culture medium were added to the wells. The disks were transferred to another new 24-well tissue culture plate after 13 h of culture, and the cell seeding efficiency was determined by counting the number of cells remaining in the wells.

After cell culturing for a week, the viability and proliferation of fibroblast cells was determined by MTT assay. The disks were rinsed with PBS three times and transferred to a new 24-well plate. Then 2 mL of culture medium were added to each well. Five μL of MTT solution (5 mg/mL) were freshly added to the culture wells and they were incubated at 37°C and 5% CO_2 for 4 h. The upper medium was removed carefully and the intracellular Formosan was solubilized by adding 2 mL of 0.04 mol/L HCl/isopropanol to each well.

The absorbance of produced Formosan was measured at 570 nm with a spectrophotometer (Shanghai Precision & Scientific Instrument Co. Ltd.). Three disks were applied for each pore size of samples. The statistical significance between two sets of data was calculated using a Student's *t* test. Data were considered to be significant when a *p* value of 0.05 or less was obtained (showing a 95% confidence limit).

Image analysis and SEM observation

A CCD-camera (type WV-CP460, Panasonic, Japan) photographed a 0.28 mm^2 field through a light microscope (Olympus IMT-2, Phase Contrast, 10× objective A10PL, Olympus photo-ocular NFK 2.5× LD). The frame grabber, installed in a compatible personal computer, digitized the information to images with 768 × 576-pixel resolution and 24-bit intensities and displayed them on a video screen. The image analysis software was provided by the Yalien Company (China).

The scaffolds, after having been cultured for 2 weeks, were taken out of the culture plates and washed with PBS three times. Thereafter, the samples were fixed with 3% glutaraldehyde in PBS for 24 h at 4°C. After being thoroughly washed with PBS, the samples were dehydrated sequentially in 50, 70, 95, and 100% ethanol each for 2–10 min. The fixed samples were freeze-dried, sputter-coated with gold, and examined under a scanning electron microscope (Hitachi S-530).

RESULTS

Porous scaffold characterization

The pore sizes of the scaffolds were calculated by analyzing the SEM pictures of the different PLLA scaffolds using the image analysis software. Figure 1 shows that pore size is related to porogen size. However, there were different degrees of reduction of pore size relative to porogen size because of the shrinkage of the polymer. Table I shows that porosity increases when the porogen weight fraction increases. The highest scaffold porosity (>90%) was obtained by modulating the proper fraction of porogen weight. A new parameter of the volume–mass index (V_p/W_s) was proposed for evaluating the scaffolds as the volume–mass index increased more than 10 times. In addition, scaffold density declined with increasing porosity.

Surface modification

It was reported that the contact angle of a typical hydrophobic surface relative to water is approximately 65–95°.[38] The average contact angle of untreated PLLA and PLGA (70/30) films are 78.0° and 70.1°, respectively. Table II shows that the hydrophilicity of both polymers was improved greatly after plasma treatment. On the other hand, it can be seen that the ratio of polar components in surface energy also increased greatly as the result of the incorporation of polar groups on the sample surface. The changes in surface composition of the samples were characterized by XPS analysis.

Figure 2 shows that a new N_{1s} peak can be observed after the NH_3 plasma treatment. The N_{1s} peak was deconvoluted into two peaks. The peak located at 399.7 eV was assigned to $-N\text{-}H^-$, and the charged nitrogen species at 401.1 eV was assigned to polaron $C\text{-}N^+$ form, suggesting that N-containing groups, for example $-NH_2$, might be incorporated into the plasma-treated surface of PLLA. The positive charged groups also could be incorporated onto the sample surface.

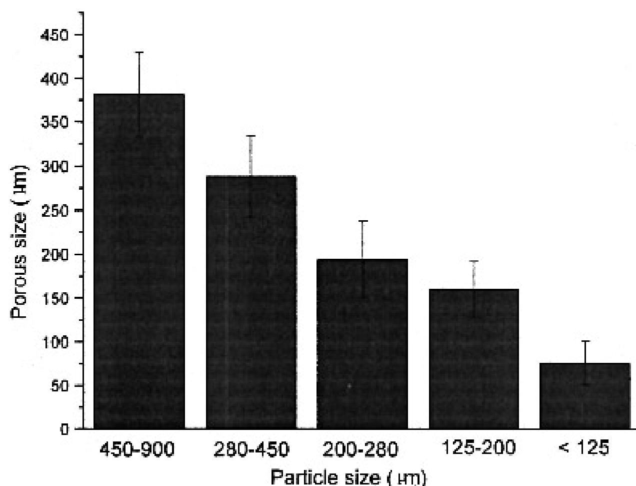

Figure 1. Relationship between porogen size and pore size of PLLA scaffolds.

TABLE I
Characterization of Porous PLLA Scaffolds

Porogen Weight Fraction (%)	Porosity of Scaffold (%)	Volume–Mass Index (V_p/W_s) (cm^3/g)	Scaffold Density $W_s/(V_p + V_s)$ (g/cm^3)
59.4	57.2	1.09	0.52
66.8	62.4	1.32	0.47
74.8	73.0	2.20	0.33
84.3	85.2	4.59	0.18
91.7	91.4	8.82	0.10
93.2	93.9	12.4	0.08

Where V_p is the volume of scaffold pores, W_s is the weight of the scaffold, and V_s is the volume of the scaffold skeleton.

On the other hand, in a comparison of two kinds of preservation methods (Fig. 3), it was found that when the plasma-treated film was preserved at room temperature, the contact angle increased greatly within 4 weeks. However, when the plasma-treated film was sealed and preserved at low temperature (0–4°C), there were almost no changes in contact angle. Therefore it is suggested that a convenient method for maintaining the plasma modification effects is to seal the modified film and then to preserve at a low temperature.

Cell culture

The cells between passages 3–8 were utilized for cell seeding. In order to understand which kinds of pores are suitable for human skin fibroblast cell culture, the mean size of the cells must first be measured. Cells were cultured in a petri dish for 4 days to allow for cell stretching. The image of the cell morphology was "grabbed" by a CCD camera and is shown in Figure 4. The size of the cells was obtained by analyzing the image through image analysis software, and the results show that the maximum length and the maximum width of the determined cells were 140 ± 40 μm and 19 ± 7 μm, respectively.

Table III shows the cell seeding loss after seeding by ethanol pre-wetting, especially for the big porous scaffold. However, for all the NH_3 plasma-treated scaffolds, the cell seeding efficiency was as high as 99%. The PLLA scaffolds were modified by NH_3 plasma treatment before the MTT assay in order to insure that

TABLE II
Changes of Contact Angle and Surface Energy After NH_3 Plasma Modification

Sample		θ (°) to H_2O	θ (°) to CH_2I_2	γ_s ($mJ.m^{-2}$)	γ_s^d ($mJ.m^{-2}$)	γ_s^P ($mJ.m^{-2}$)	X^P ($mJ.m^{-2}$)
PLLA	control	78.0	37.0	43.2	32.5	10.7	0.25
	modified	21.5	40.0	68.9	26.7	42.2	0.61
PLGA (70/30)	control	70.1	37.2	45.9	31.0	14.9	0.32
	modified	15.9	33.4	71.7	29.1	42.6	0.59

γ_s: surface energy; γ_s^d: dispersive components; γ_s^P: polar components; $X^P = \gamma_s^P/\gamma_s$. Parameters of plasma treatment are 50 w, 20 Pa, and 120 s.

the amounts of the cells seeded into the scaffolds were equal. The MTT assay (Fig. 5) indicated that higher absorbance was obtained when the pore size was less than 160 μm (mean value).

For scaffolds D and E, there is statistical significance ($p < 0.05$) compared with scaffolds A, B, and C. There is no statistical significance between scaffolds D and E.

The morphology of fibroblast cells cultured on porous PLGA (70/30) scaffolds was observed by SEM, as shown in Figure 6. For the untreated PLGA scaffold, it can be seen that there were only a few cells in the pores, as shown in Figure 6(C), with many cells just crawling along the edge of the pores. However, for the NH_3 plasma-treated PLGA scaffolds [Fig. 6(D)], the cells migrated into the pores and adhered to the wall of the pores through the filopodial extensions. The metabolism of the cells was vigorous and a great deal of extracellular matrix (ECM) was secreted. However, another phenomenon found in the middle of the scaffold [Fig. 6(E)] cannot be neglected. Some cells adhered to the wall of the pores, but the morphology was not satisfactory.

Figure 6(F) shows that the cells proliferated promptly on the plasma-treated PLGA scaffold and the ECM secreted by the cells covered some pores of the scaffolds.

DISCUSSION

A particulate-leaching technique was a convenient method for forming the sponge-like scaffolds. It was suitable for forming macroporous polymer scaffolds, and the pore size and porosity of the scaffold could be easily controlled. For specific tissue engineering applications, different pore structure scaffolds were needed, varying for different cell types. In this study, the different pore structures of PLLA and PLGA (70/30) cell scaffolds were fabricated and characterized.

Generally, there are two methods to determine porosity: mercury intrusion porosimetry and liquid displacement by measuring the volume.[35] The former method must proceed in a closed system because of the toxicity and volatility of mercury, and the procedures also are complex and dangerous. With the latter method the investigator must assure that there is no loss of ethanol when repeating the evacuation-repression cycles.

It is difficult to do this using a graduated cylinder. Therefore, for this study an improved method, based on the Archimedes' Principle, was developed. A density bottle was used instead of the graduated cylinder. The advantages of this method are the avoidance of using toxic mercury and the assurance of a more accurate weight measurement than can be achieved with

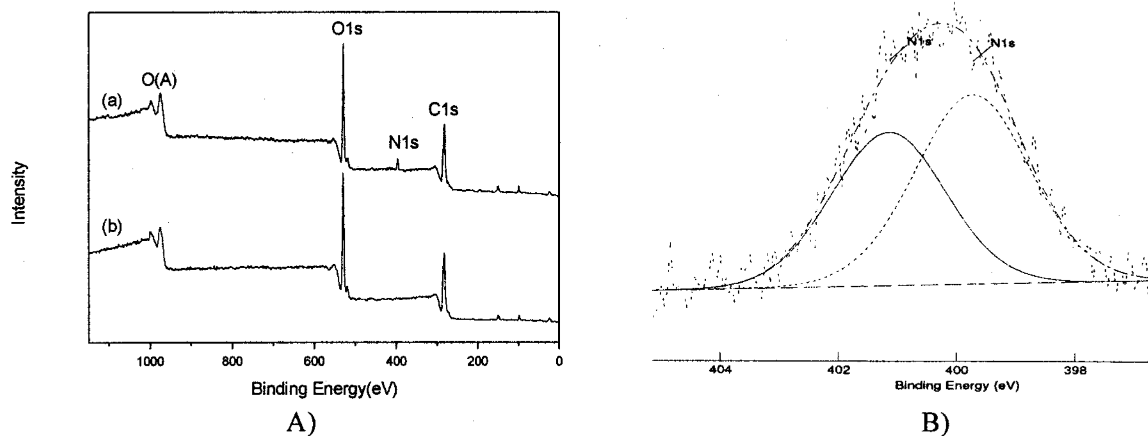

Figure 2. Surface composition analysis of PLLA films by XPS: (A) survey spectra of (a) plasma modified and (b) control; (B) deconvoluted N_{1s} spectra of ammonia plasma-modified samples.

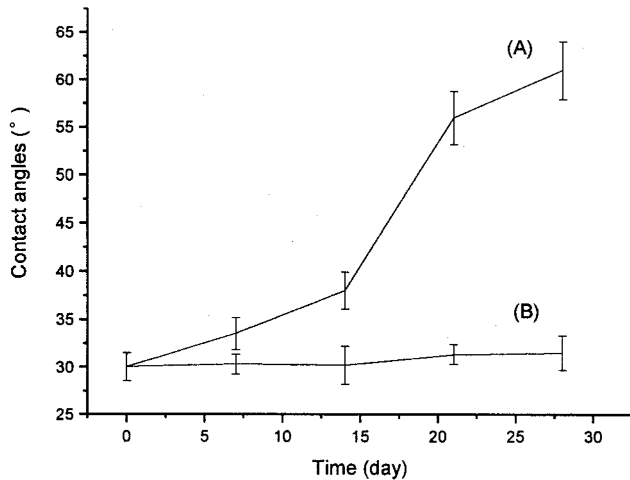

Figure 3. Dependence of water contact angle of plasma-modified PLLA films on preserving time: (A) at room temperature in desiccator; (B) sealed and preserved at 0–4°C.

methods that measure the volume directly. The air bubbles in the scaffold could be removed easily under vacuum without worrying about the loss of the liquid. In addition, a new parameter, the volume–mass index, was proposed. It was very suitable for characterizing changes in the morphology and strength of a scaffold.

As with other synthetic materials, there are no natural recognition sites of cells on the surfaces of PLLA and PLGA.[39] Hydrophobicity and low surface energy affect cell migration into the pores and also affect cell attachment and growth on the polymer. Since there is an absence of functional groups in the backbone of PLLA and PLGA, it is hard to modify by common chemical methods the surface properties of the polymers, especially for a scaffold of complex structure. The plasma technique, however, is a convenient method for modifying surface properties, including the complex shape of a porous scaffold because it is

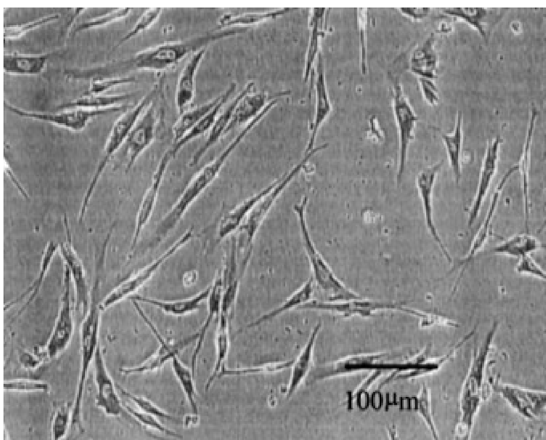

Figure 4. Human skin fibroblast cells cultured in Petri dishes for 4 days.

TABLE III
Cell-Seeding Efficiency of PLLA Scaffolds of Different Pore Sizes

Scaffold Processing Method	Scaffold Pore Size (μm)	Cell-Seeding Efficiency (%)
NH_3 plasma treatment	381 ± 48	99.20 ± 0.19
	288 ± 46	99.19 ± 0.14
	194 ± 44	99.38 ± 0.12
	160 ± 32	99.43 ± 0.01
	76 ± 25	99.54 ± 0.04
Wetting by ethanol	381 ± 48	80.47 ± 4.06
	194 ± 44	87.38 ± 2.29
	76 ± 25	98.95 ± 0.09

conducted under vacuum and it tends to be pervasive.[27]

After anhydrous ammonia plasma treatment, the hydrophilicity and surface energy were greatly improved (Table II). Positive charged groups also appeared on the surface of the materials. On the other hand, the disadvantage of plasma treatment is that the modifying effects of the materials are partially lost when the surface undergoes reconstruction.[40] In our experiments, however, we found that the modifying effects could be maintained by preserving the modified samples at a low temperature (0–4°C) with no apparent changes in the contact angles of the samples. Considering the Tg of PLLA and PLGA were 55.0°C and 45.6°C, respectively, the preservation temperature of 0–4°C was much less; thus the mobility of the surface molecular chains was nearly frozen. As a result, the hydrophilicity could be kept unchanged. This preservation method also could be applied to other plasma-treatment devices.

For a specific cell type, a proper pore size benefits

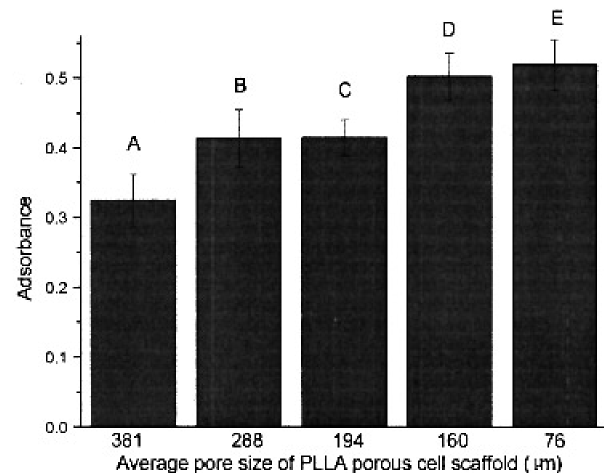

Figure 5. MTT–tetrazolium assay of human skin fibroblast cells cultured in various PLLA scaffolds for 1 week. Formosan absorbance is expressed as a function of the pore size of PLLA scaffolds: $P < 0.05$ for D or E vs. A, B, and C; $P > 0.05$ for D vs. E.

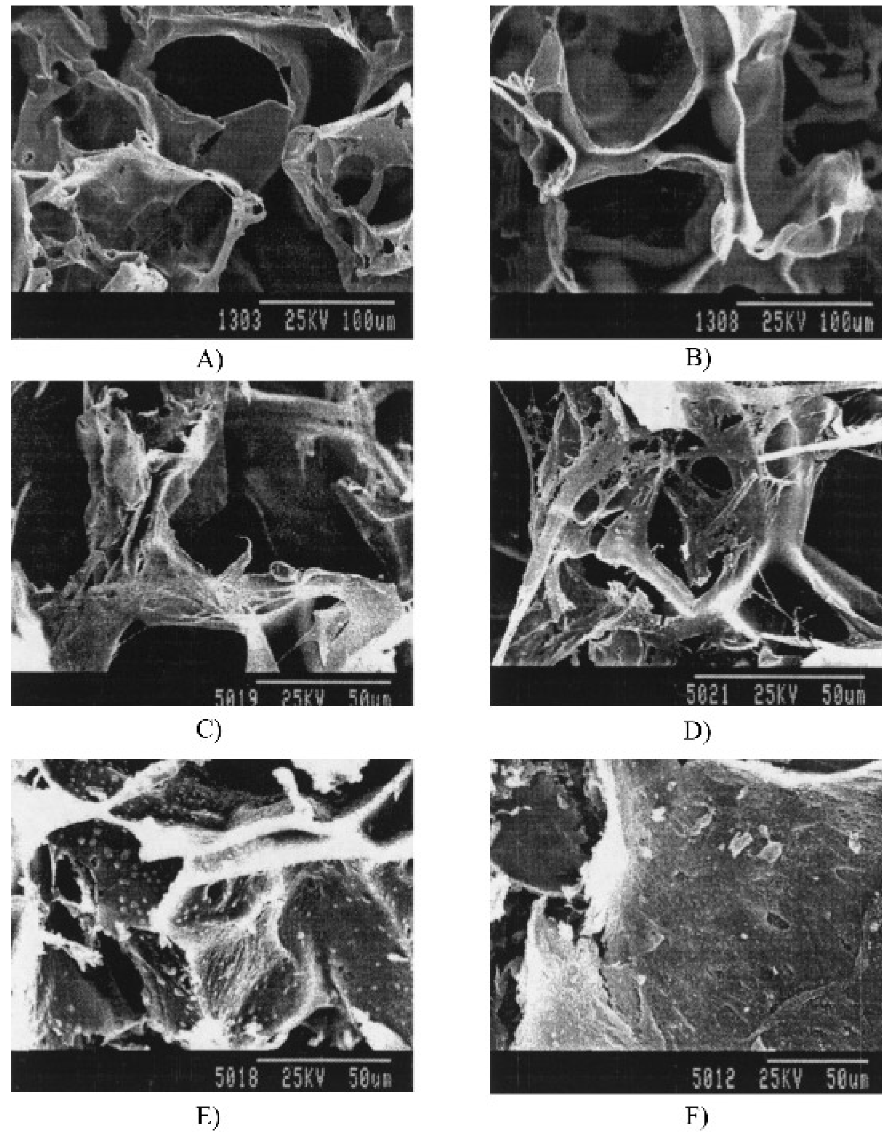

Figure 6. SEM images of the PLGA (70/30) scaffolds before and after human skin fibroblast cells culture for 2 weeks: (A) surface of scaffold; (B) cross section of scaffold; (C) cross section of untreated scaffold after cell culture; (D) cross section of plasma-treated scaffold after cell culture; (E) some pores located at the middle part of plasma-treated scaffold after cell culture; and (F) surface of plasma-treated scaffold after cell culture.

cell attachment and growth. The MTT assay showed that more viable cells existed in scaffolds D and E (<160 μm) after 1 week of culture, making them quite suitable for human skin fibroblast cell growth. This is in agreement with the size of human skin fibroblast cells in a previous study.[21]

A large number of cells can be achieved by cell culture *in vitro* in tissue engineering. However, it is tedious work and cell loss often occurs during cell seeding. Therefore it is important to increase cell-seeding efficiency in tissue engineering. Anhydrous ammonia plasma treatment can assure that cell seeding efficiency is maintained as high as 99%. In addition, after plasma treatment, the hydrophilicity of scaffolds is improved and the scaffolds remain in a dry state, accommodating the cell suspension inside the scaffold after the cell suspension infiltrates into the pores promptly.

If the scaffolds are pre-wetted by ethanol, the scaffold must be washed by PBS solution and the residual solution in the pores must be removed by a micropipette. If the residual solution in the pores cannot be removed completely, it will repel the entrance of the cell suspension. If too much water evaporates, the effects of wetting the surface will not be achieved. It is not easy to control the degree of surface wetting. In addition, the problem of toxicity of ethanol trace and hydrophobicity of PLGA surfaces are still remains.[41] Plasma treatment, on the other hand, can be easily controlled, contributes to the maintenance of high cell-seeding efficiency, and helps avoid the negative effects of residual ethanol on cell culture.

The SEM results demonstrate that the anhydrous ammonia plasma treatment can improve cell attachment and cell growth effectively. This may be the case because adhesive glycoproteins, such as fibronectin and vitronectin, play important roles in the initial cell attachment.[42,43] Also, surface energy might play a role in attracting particular proteins to the surface of the scaffold and, in turn, improving the affinity of the cells to the scaffolds.[44] It is possible that the improved surface energy affects the adsorption of particular glycoproteins. On the other hand, the incorporated N-containing groups are capable of efficient interaction with proteins by hydrogen bonding, which might affect the adsorption of serum adhesive glycoproteins. In addition, the positive charged groups also enhance the interaction between the surface of materials and the cells that carry a negative charge.

Another issue, one related to mass transport, cannot be neglected. It has been reported that chondrocytes, with their low metabolic activity, can survive several millimeters from a blood supply. But high metabolic activity cells, such as fibroblasts and hepatocytes, cannot be engineered with a thickness greater than several hundred microns.[9] For the plasma-modified PLGA scaffolds, with a thickness of 1.48 mm, cells proliferated promptly and produced a great deal of ECM, which filled in the pores. In addition, on the surface of the scaffold some pores were covered by the ECM produced by the cells [shown in Fig. 6(F)]. For these two reasons, the nutrient transport must be restricted, which would affect the nutrient supply of the cells in the middle part of the scaffolds. Mass transport is one of the most significant challenges in tissue engineering. Since the body's capillary networks are charged with nutrient transport, it will be necessary to promote angiogenesis for the formation of tissues in order to insure tissue longevity and viability. The perfusion culture[45] also might help to resolve this problem.

CONCLUSIONS

Porous PLLA and PLGA (70/30) cell scaffolds with different pore structures were fabricated by a particulate leaching method. An improved method based on the Archimedes' Principle was developed for measuring the porosity of the scaffolds, and a new parameter (V_p/W_s) for characterizing the changes in the morphology of the scaffolds was proposed. The scaffolds were modified by an anhydrous ammonia gas plasma treatment and characterized relative to hydrophilicity, surface energy, and surface composition. The results show that the hydrophilicity and surface energy of the scaffolds improved greatly. The positive charged groups also appeared on the materials' surfaces. A convenient low-temperature preservation method was proposed for maintaining the effects brought about by the treatment. Human skin fibroblast cells were used to evaluate the cell scaffolds. The results show that pores smaller than 160 μm were suitable for human skin fibroblast cell growth. Anhydrous ammonia plasma treatment enhanced e cell affinity and cell seeding efficiency, which was able to be maintained at above 99%. The negative effects of the residual ethanol on cell culture were avoided by utilizing plasma treatment. Mass transport issues also were discussed and some suggestions were proposed.

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